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BRCAness: Finding the Achilles Heel in Ovarian Cancer

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Abstract

Ovarian cancer is the leading cause of death among gynecological cancers. It exhibits great heterogeneity in tumor biology and treatment response. Germline mutations of DNA repair genes *BRCA1/2* are the fundamental defects in hereditary ovarian cancer that expresses a distinct phenotype of high response rates to platinum agents, improved disease-free intervals and survival rates, and high-grade serous histology. The term "BRCAness" describes the phenotypic traits that some sporadic ovarian tumors share with tumors in *BRCA1/2* germline mutation carriers and reflects similar causative molecular abnormalities. *BRCA* pathway studies and molecular profiling reveal *BRCA*-related defects in almost half of the cases of ovarian cancer. *BRCA*-like tumors are particularly sensitive to DNA-damaging agents (e.g., platinum agents) because of inadequate *BRCA*-mediated DNA repair mechanisms, such as nucleotide-excision repair and homologous recombination (HR). Additional inhibition of other DNA repair pathways leads to synthetic lethality in HR-deficient cells; this has been employed in the treatment of *BRCA*-like ovarian tumors with poly(ADP-ribose) polymerase inhibitors with promising results. This article presents a comprehensive review of the relevant literature on the role of BRCAness in ovarian cancer with respect to *BRCA* function, methods of *BRCA* epigenetic defect detection and molecular profiling, and the implications of *BRCA* dysfunction in the treatment of ovarian cancer. *The Oncologist* 2012;17:956–962

Introduction

Ovarian malignancies are a group of heterogeneous tumors that express diverse pathologic characteristics and biological behavior. Hereditary ovarian cancer comprises 10%–15% of all cases of ovarian malignancies and is mainly associated with germline mutations in the *BRCA1* and *BRCA2* DNA repair genes [1]. Ovarian tumors in *BRCA*-mutated patients have relatively uniform behavior with high overall response rates to first-line platinum-based treatment [2–4], high response rates to platinum-based chemotherapy at first and subsequent relapses [2, 3], long disease-free intervals [2–4], improved overall survival rates (especially in more advanced stages) [2–5], possibly higher incidences of visceral metastases [6], and usually (but not exclusively) high-grade serous histology [5–9]. In a multivariate analysis for independent predictors of survival, *BRCA* status was one of three parameters (together with patient age and extent of surgery) associated with patient survival rates both in the subgroup of patients with stage III disease and in the entire study population [2].

The term "BRCAness" has been used to describe the phenotypic characteristics that some sporadic ovarian cancers share with tumors found in the setting of *BRCA* germline mutations. The term also reflects that this common biologic behavior comes from molecular defects in the cellular machinery similar to the ones caused by *BRCA* mutation [10, 11]. The notion began to form in 1996 after studies of *BRCA1/2* genes in sporadic ovarian cancer showed multiple defects in the *BRCA1/2* pathway that would explain a *BRCA*-like phenotype [12–17].

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Table 1. Some molecular defects that can lead to BRCAness

Defective mechanism	% in ovarian cancer
BRCA1/2 germline mutation	10–15
BRCA1/2 somatic mutation	5–10
BRCA promoter methylation	5–30
EMSY amplification	20
Fanconi anemia complex defects	21
PTEN focal deletion/mutation	7
Rad51C hypermethylation	3
ATM/ATR mutation	2

BRCA Gene and Molecular Defects in BRCAness

The phenotypic traits of BRCAness are reflective of defective function of the *BRCA* pathway in the affected cancer cells (Table 1). The *BRCA1* and *BRCA2* tumor suppressor genes are implicated in cell proliferation, DNA damage response, and DNA repair. DNA is under constant stress during replication, transcription, and exposure to harmful agents such as ionizing radiation, oxygen radicals, and genotoxic chemical compounds including antitumor drugs. When DNA damage occurs, sensory proteins, such as the kinases ATM and ATR that participate in cell cycle checkpoints, activate DNA repair pathways that vary according to the kind and extent of the damage inflicted [18]. Knowledge of DNA damage response pathways and their status in cancer facilitates prediction of the sensitivity of healthy and neoplastic tissues to chemotherapeutic agents and radiation and permits exploitation of the defects of these pathways in favor of the patient.

BRCA1 and *BRCA2* germline mutations are the fundamental defect in hereditary ovarian cancer where the normal allele of the carrier is inactivated in cancer cells [17, 19]. On the contrary, *BRCA1/2* somatic mutations are generally rare in the sporadic forms [12, 15, 17, 20–22] but still are a significant causative gene defect as shown in extensive genomic analyses of ovarian carcinoma by the Cancer Genome Atlas Research Network [23]. Higher incidence of somatic mutations is found in patients with specific characteristics, such as Italian or Jewish origin, serous histology, and younger age [24]. Either genetic or somatic mutations of *BRCA1* and *BRCA2* are found in approximately 20% of all ovarian tumors [16]; *BRCA1/2* alterations of all kinds, including mutations, have been reported in up to 82% of ovarian tumors [17].

In cases other than *BRCA* mutations, the BRCAness pattern of biological and clinical behavior seems to be the result of different epigenetic processes. The *BRCA1* promoter aberrant methylation in cytosine residues of CpG dinucleotides has been shown to lead to decreased *BRCA1* expression in 5%–30% of ovarian tumors, resulting in BRCAness [13–15, 23]. A subsequent study indicated that *BRCA1* promoter methylation can be a particularly adverse prognostic factor compared to either *BRCA1* germline mutation or no loss [25]. A more recent report found epigenetic silencing of *BRCA1* and *BRCA1/2* mutations to be mutually exclusive; patients with epigenetic *BRCA1* silencing were found to have similar prognosis with wild-type carriers [23]. Although loss of heterozygosity for the *BRCA* locus has been noted in sporadic breast cancer [26], the importance of this mechanism has not been verified in ovarian cancer.

BRCAness could also emerge from defects in genes whose function either affects or is affected by normal *BRCA* gene function. A typical example is the amplification of *EMSY* that leads to *BRCA* silencing. The *EMSY* gene is amplified in about 20% of cases of high-grade serous ovarian carcinomas [27] and disrupts *BRCA2* participation in DNA damage response, rendering the cell prone to genomic instability [28, 29]. *BRCA* cooperates with the proteins of the Fanconi anemia (FA) complex in the pathway of DNA repair and thus defects in members of the FA complex reproduce the *BRCA*-deficient phenotype [30]. Methylation of the FA complex gene *FANCF* is found in 21% of ovarian cancers and ovarian cell lines with *FANCF* methylation demonstrated high sensitivity to platinum agents that was reversed with *FANCF* demethylation [31].

Defects in proteins involved in DNA repair besides *BRCA* could theoretically also lead to BRCAness. In a large-scale genomic analysis of ovarian cancer cases, hypermethylation of Rad51C, a protein that locates DNA repair machinery to the damaged strand, was found in 2% of the cases. In addition, mutations of the DNA-damage sensory proteins ATM and ATR were found in 3% of the cases [23]. In the same study, *PTEN* was deleted or mutated in 7% of the cases [23]. *PTEN* is involved in transcription regulation of Rad51 and genomic integrity maintenance [32]; a *BRCA*-like phenotype could emerge when the function of either one of these is disrupted.

BRCA Defect Detection and Molecular Profiles

Screening for mutations is impractical for large populations and also not informative for other kinds of defects in the *BRCA* pathway that can lead to BRCAness. Loss of heterozygosity (LOH) caused either by a germline mutation or an epigenetic change may be a better way to identify tumors that behave in a *BRCA*-like way. In one study, the presence of LOH was frequently associated with *BRCA* somatic or germline mutation, especially in the presence of family history [15]. In another study, immunohistochemistry for *BRCA1* demonstrated a sensitivity of 80%, specificity of 93%, and positive predictive value of 73% for detecting a *BRCA1* mutation [33]. Of note, high *BRCA1* protein expression detected with immunohistochemistry had a negative prognostic value for progression-free survival in patients with ovarian cancer and minimal residual disease [34]. *BRCA1* loss was assessed in breast cancer via a comparative genomic hybridization classifier; a positive result had positive predictive value for the efficacy of DNA damage-inducing chemotherapy [35]. Certain morphologic patterns in tumor specimens may predict for a *BRCA*-defective genotype. More specifically, the *BRCA1/2* genotype in high-grade serous ovarian carcinoma was found to be associated with solid, pseudo-endometrioid, or transitional cell carcinoma-like mor-

phology; higher mitotic indices; increased tumor-infiltrating lymphocytes; and necrosis. In fact, these characteristics could predict its presence with 100% sensitivity and 57% specificity [36]. *BRCA2*-mutated tumors seem to have necrosis and tumor-infiltrating lymphocytes to a lesser extent than those with a *BRCA1* mutation [36], although a study in a much greater population showed no statistically significant differences between *BRCA1*- and *BRCA2*-mutated ovarian tumor pathology [37].

An early attempt to describe the BRCAness pathway explored the molecular profiles of nonredundant, significantly expressed genes of *BRCA1*- and *BRCA2*-mutated ovarian tumors and then used them to segregate sporadic cancers in two *BRCA1* or *BRCA2*-like groups [38], suggesting that *BRCA1*-like and *BRCA2*-like molecular profiles are expressed in some sporadic ovarian tumors. More recently, a BRCAness gene signature was developed from samples of *BRCA1/2*-mutated tumors, which successfully predicted platinum responsiveness in tumor specimens. The presence of a BRCAness profile also carried strong independent prognostic value for patients with sporadic ovarian cancers [39]. Interestingly, in these studies the gene signatures of sporadic *BRCA*-like tumors were much more like those of *BRCA* tumors than non-*BRCA*-like sporadic cancers. Additionally, a *BRCA*-like profile was associated with longer survival times. In fact, there were some hereditary *BRCA* tumors that expressed a signature similar to the nonhereditary, non-*BRCA*-like tumors, whereas the *BRCA*-like tumors clustered with most hereditary *BRCA* tumors. Furthermore, there were more similarities between *BRCA1* and *BRCA1*-like tumors and between *BRCA2* and *BRCA2*-like tumors, respectively, than between *BRCA1* and *BRCA2* tumors [39].

Of note, the primary *BRCA* defect in *BRCA*-deficient tumors may correlate with alterations of the molecular profile. *BRCA* genetic loss relates to decreased *PTEN* mRNA levels, whereas epigenetic loss of *BRCA1* is related to copy number gain of *PIK3CA* [40]. It is well known that both of these defects lead to the activation of the PI3K/Akt pathway. Also, accumulation of mutated p53 protein, which is the most common somatic genetic event in ovarian cancer, was found in the same frequency in *BRCA1/2* mutated and nonmutated cases [41]. However, overexpression of p53 with loss of p21 expression is significantly more frequent in high-grade serous carcinomas with epigenetic loss of *BRCA1* compared with high-grade serous tumors without loss of *BRCA1* or with *BRCA1* somatic and germline mutations [40].

BRCAness in Treatment

BRCA1 and *BRCA2* are mainly involved in the path of homologous recombination (HR) that repairs DNA interstrand crosslinks and double-strand breaks [42]. *BRCA1* also participates in nonhomologous end joining of double-strand breaks and nucleotide excision repair of DNA adducts [43]. Double-strand breaks and DNA adducts are the typical DNA damage caused by DNA alkylating agents such as cisplatin and mitomycin. *BRCA*-deficient cells are highly sensitive to these agents in vitro [44, 45]. Many clinical studies in patients with *BRCA*-deficient ovarian cancer have demonstrated high sensitivity of these tumors to platinum-based therapy [3, 11, 46] that leads to long disease-free intervals and improved overall survival rates [2–5, 9]. A recent study of women with high-grade serous ovarian cancer revealed significant chemosensitivity and survival benefit only in *BRCA2* mutation carriers compared with *BRCA1* mutations and *BRCA1/2* wild types [47], but the study received strong criticism for its low statistical power. Nonetheless, these conflicting results stress the need for evaluation of BRCAness status as a stratification factor in large phase III studies, especially in light of new targeted therapies.

BRCA status also appears to affect the efficacy of mitotic spindle poisons, such as the taxanes. *BRCA1* participates in the mitotic checkpoint at the metaphase-anaphase transition and controls the proper segregation of chromosomes between daughter cells [48, 49]. Spindle disruption leads to apoptotic cell death that involves the *JNK* pathway [50]. *BRCA1* activates the *JNK* pathway [51]; in *BRCA*-deficient breast cancer cells, paclitaxel treatment led to reduced *JNK* activation and lower apoptosis [50]. These findings suggest that *BRCA1* directs cells towards apoptotic death after spindle poison-based treatment, in contrast to its protective role in DNA repair and cell survival after treatment with DNA-damaging treatment.

Studies in breast cancer cell lines showed that loss of *BRCA1* function leads to taxane resistance [52, 53], but a clinical study showed that decreased expression of *BRCA2* mRNA predicted a favorable response to docetaxel in breast cancer [54]. PI3K/Akt activation in *BRCA*-deficient ovarian cancer [40] could also contribute to taxane-resistance as overexpression of activated *AKT* has been shown to decrease apoptosis induced by paclitaxel in ovarian cancer cells [55]. Overall, there have been conflicting reports on the role of *BRCA* in taxane-sensitivity in ovarian cell lines [56–58] with the latest showing that inhibition of endogenous *BRCA1* expression results in increased sensitivity to platinum therapy and decreased sensitivity to spindle poisons. In the same report, high *BRCA1* mRNA expression levels were associated with increased overall survival rates for ovarian cancer after taxane-containing chemotherapy. This *BRCA*-related binary behavior could be used in treatment planning for ovarian cancer.

Poly(ADP-Ribose) Polymerase Inhibitors

BRCA-deficient cells have defective HR capacities and are thus dependent on other pathways to repair DNA damage. The interruption of those pathways is likely to be deleterious for those cells, while leaving cells with adequate HR function unaffected. This is the thinking behind synthetic lethality—a term referring to the targeted exploitation of genes relating to functions that are already defective in a particular cell. The two insults together are lethal for the cell, whereas cells with one or the other defect remain unaffected [59, 60]. The term was first used in 1946 by Dobzhansky in *Drosophila* studies [61].

BRCA-deficient cancers are ideal targets for synthetic lethality, which has been accomplished by targeting another DNA repair pathway called base excision repair (BER) through poly(ADP-ribose) polymerase (PARP) inhibition. PARPs are a family of enzymes that play a key role in the re-

pair of single-strand breaks through BER. PARP1 is the most abundant member of this family and is the main target of a novel category of molecules called PARP inhibitors [62]. PARP inhibition causes accumulation of DNA single-strand breaks, which, when left unrepaired, lead to potentially lethal double-strand breaks. In normal cells, the latter can be repaired through HR. However, in *BRCA*-deficient cells, the combined inadequacy of HR and BER leads to cell death—a typical example of synthetic lethality [63, 64].

In a very impressive example of translational research, PARP inhibitors were rapidly taken to phase I studies demonstrating considerable antitumor activity against *BRCA1/2*-related tumors of ovarian, breast, and prostate origin with acceptable toxicity [65, 66]. The following proof-of-concept phase II studies of the PARP1 inhibitor olaparib [67] in *BRCA1/2*-mutated patients with advanced chemorefractory breast cancer and recurrent platinum-treated ovarian cancer [68, 69] showed dose-related response rates and good tolerability.

In concordance with the BRCAness theory, PARP inhibition proved to be synthetically lethal for cells lacking other proteins involved in homologous recombination besides *BRCA1/2*, such as RAD51, ATR, ATM, CHK1, and FANCA or FANCC [70]. This is important because homologous recombination seems to be defective in almost half of ovarian cancers [23]. Olaparib was tested as monotherapy in a phase 2 study in patients with high-grade serous and/or undifferentiated ovarian cancer or triple-negative breast cancer. Patients were stratified according to *BRCA* status; high response rates in both *BRCA*-mutated and nonmutated ovarian tumors were observed. However, the same study failed to show any benefit for patients with triple-negative breast cancer [71].

Similar outcomes were reported for a phase III study of the addition of iniparib to gemcitabine and carboplatin treatment for patients with triple-negative breast cancer. That study failed to show significant improvement in the coprimary endpoints of overall and progression-free survival rates, although patients receiving iniparib as second- or third-line treatment had a modest but still not significant benefit [72]. However, the results of this study could be misleading; iniparib's primary mechanism of action may be the modification of cysteine-containing proteins and not PARP inhibition [73]. Furthermore, iniparib failed to kill homologous recombination-deficient cells or inhibit PARP activity in vitro compared with better characterized PARP inhibitors such as olaparib in a recent study [74].

Most recently, olaparib was found to have equivalent efficacy with liposomal doxorubicin in patients with recurrent *BRCA1/2* mutated ovarian tumors [75]. It must be noted that the outcome in the liposomal doxorubicin arm of this study was significantly superior to what would be anticipated based on historical data; this may be coincidental, but it could also be due to the increased sensitivity of *BRCA*-mutated tumors to DNA poisons.

The first explanation for the conflicting results of PARP inhibition between breast and ovarian *BRCA*-mutated tumors that comes to mind is that "triple negativity" is not an appropriate surrogate marker for BRCAness [76] in breast cancer. On the other hand, BRCAness seems to be fairly well defined and convincingly documented in ovarian cancer. However, better markers of BRCAness are still needed, especially in the light of potential therapeutic gain from PARP inhibition.

A list of ongoing trials of PARP inhibitors in ovarian cancer can be found on the site www.clinicaltrials.gov. Searching for the terms "PARP inhibitors" and "ovarian cancer" recalls 28 studies, of which six are completed. Results have been published for four of the completed studies [65, 69, 71, 75]. The other two completed trials of PARP inhibitors in ovarian cancer are a single-arm study of iniparib in patients with *BRCA1/2*-associated ovarian cancer (ClinicalTrials.gov identifier NCT00677079) and a phase I study of the PARP inhibitor veliparib in combination with temozolomide in patients with various cancer types, including ovarian cancer (NCT00526617). The PARP inhibitor olaparib is used in eight of the ongoing studies, administered either alone, with chemotherapeutic agents (in combination or sequentially with carboplatin with or without paclitaxel) or with antiangiogenic agents (cediranib) in *BRCA*-deficient or sporadic ovarian cancer.

The PARP inhibitor veliparib is studied in five phase I trials that include patients with ovarian cancer, given alone or with irinotecan, topotecan, pegylated liposomal doxorubicin, carboplatin, paclitaxel, bevacizumab or temozolomide, as well as four phase II studies with pegylated liposomal doxorubicin, topotecan, temozolamide and cyclophosphamide. Iniparib is being studied in three phase II studies of recurrent ovarian cancer—as a single agent in one study and in combination with gemcitabine/carboplatin in the other two studies. Two more trials are investigating PARP inhibitors AGO14699 (phase II) and MK4827 (phase I) in ovarian cancer [77]. Data on clinical trials was current as of April 10, 2012, updated from [77]. Results from these trials should clarify the role of PARP inhibitors in ovarian cancer and the need to identify *BRCA*-like cases.

What Happens After PARP Inhibition?

Tumor behavior after PARP inhibition therapy is also interesting. Preliminary analysis of olaparib-treated patients with chemorefractory ovarian cancer showed remarkable response to carboplatin and/or paclitaxel-based treatment after disease progression, although a causative relationship between olaparib and the subsequent enhanced chemosensitivity cannot be established [78]. Finely targeted therapy provokes critical, equally precise resistance mechanisms in the constantly changing cancer cell.

A report from 2008 revealed a reversion of *BRCA2* mutation in *BRCA2*-mutated platinum-sensitive ovarian cancer as a platinum resistance gaining mechanism [79]; similar restoring mutations in both *BRCA1* and *BRCA2* were recently described in primary and recurrent patients with *BRCA1/2*-mutated ovarian cancer who had previously received chemotherapy. These mutations appeared in 28% of recurrent ovarian carcinomas and 46% of the platinum-resistant cases and were predictive of platinum chemotherapy resistance [80, 81].

Reversion of the *BRCA2* mutation also confers resistance

to PARP inhibition therapy [82]. However, PARP inhibitor resistance can potentially occur through other mechanisms as well, such as upregulation of other DNA repair pathways, activation of cell proliferation pathways, or mutations in the *PARP* gene that alter the interaction of PARP with its inhibitor [83]. As it seems, *BRCA*-deficient ovarian cancer would be platinum-sensitive and taxane-resistant, so under the pressure of platinum-based therapy a *BRCA*-restoring mutation in these cells could reverse the sensitivity-resistance relationship, perhaps rendering the cells taxane-sensitive again. Alternating *BRCA* function could be of use in optimizing therapeutic gain from variants of taxane/platinum regimens [84].

In an attempt to predict sensitivity to PARP inhibition, si-RNA screen studies showed that defects in genes involved in DNA repair pathways other than HR can be used with PARP inhibition for synthetic lethality and revealed novel targets such as CDK5, MAPK12, PLK3, and the transcription coupled DNA repair proteins DDB1 and XAB2–9 [85, 86]. More recently, *BRCA1* CpG island hypermethylation was also proposed as another predictive factor of PARP inhibition sensitivity [87]. Rad51 nuclear foci, which are formed when *BRCA1* senses DNA double-strand breaks, were studied as a marker for adequate HR in ovarian cancer samples and correlated with in vitro response to PARP inhibition [88].

Synthetic lethality in *BRCA*-deficient tumors could be achieved with other DNA repair-disrupting molecules. ATM inhibition in FA pathway-deficient cells was shown to result in DNA breakage, cell cycle arrest, and apoptotic cell death [89]. Furthermore, ATM kinase inhibitor use sensitized mantle lymphoma cells to PARP inhibitors [90], underlining the potential of multitargeted DNA repair inhibition in already DNA-repair defective tumors. Decreased PTEN levels in *BRCA*-defective cells could enhance synthetic lethality with PARP inhibition, as PTEN-deficient cells were 20-fold more sensitive to PARP inhibition and showed decreased levels of Rad51 foci formation [32, 91, 92].

FOXM1 transcriptional factor network is upregulated in 84% of high-grade serous ovarian cancer [23] and is related to tumorigenesis and tumor proliferation [93]. FOXM1 crosstalks with the *BRCA* pathway [94] and could represent a novel therapeutic target in ovarian cancer. The role of hypoxia in this setting should also be assessed because the latter inhibits DNA repair mechanisms [95] and has been reported to lead to decreased expression of *BRCA1/2* and *Rad51* [96, 97] and, therefore, to increased PARP inhibition sensitivity in tumor cells [98].

Summary

Ovarian cancer is the fifth most common female cancer in the western world. *BRCA1/2* germline mutations are the most common defect that gives rise to hereditary ovarian cancer, accounting for about 10% of cases. Further study implicated these genes in sporadic ovarian cancer via multiple deactivating mechanisms that all lead to impaired function of *BRCA1/2* and thus to a distinct phenotype called BRCAness. The central role of *BRCA* in DNA damage response and repair renders *BRCA*-defective cells sensitive to DNA damaging agents. Further inhibition of other DNA repair pathways can be deleterious for *BRCA*-defective cells. This synthetic lethality is employed in new targeted treatments in ovarian cancer, such as PARP inhibition, thus leading to the best example of personalized therapy in ovarian cancer to date.

Improved *BRCA* defect characterization and detection will allow better patient selection and possibly improved clinical outcomes for a disease that is still the leading cause of death among gynecological malignancies. This type of extensive and in-depth rational understanding of a biological variant is rare and warrants further development—not only for its obvious, inherent therapeutic implications, but also as a model of studying and understanding core processes in cancer cells.

Author Contributions

Conception/Design: Georgios Rigakos, Evangelia Razis
Provision of study material or patients: Georgios Rigakos, Evangelia Razis
Collection and/or assembly of data: Georgios Rigakos, Evangelia Razis
Data analysis and interpretation: Georgios Rigakos, Evangelia Razis
Manuscript writing: Georgios Rigakos, Evangelia Razis
Final approval of manuscript: Georgios Rigakos, Evangelia Razis

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